Evolution of composition of dairy manure supernatant in a controlled dung pit.

C. Rico[a,*], H. García[b], J.L. Rico[b], J. Fernández[b], J. Renedo[b]

[a] Department of Sciences and Techniques of Water & Environment, University of Cantabria, Spain. Los Castros s/n, 39005 Santander, Spain.
[b] Department of Chemical Engineering and Inorganic Chemistry, University of Cantabria, Spain. Los Castros s/n, 39005 Santander, Spain.

Abstract

Anaerobic conversion of dairy manure into biogas is an attractive way of managing this waste. It is well known that the hydrolysis of large molecules into small, directly biodegradable ones, is the rate limiting step of the overall anaerobic process. The present work studies the development of the hydrolytic and acidogenic stages of dairy manure with different solid concentrations (40, 60 and 80 g VS/l) at ambient temperature (20ºC). The purpose was to determine the operational conditions that provide a liquid fraction with high soluble COD and Volatile Fatty Acids (VFA) contents in manure before methanogenic stage starts up. At 20ºC, the evolution of the studied parameters showed that in a controlled plug flow dungpit, hydrolytic and acidogenic stages progressed moderately in a continuous way during the 25 days that the experimentation lasted, whereas no methanization was observed. Supernatant COD and VFA concentrations increased 30% and 107% respectively for the 60 g VS/l samples. Manure was also operated at 35ºC with a similar increment in supernatant COD but a higher increase in VFA, 154%. For both operational temperatures the majority VFA were, in this order, acetic, propionic and butyric. During the operation at 35ºC, methanogenic stage started between days 20 and 25 for the samples with lower solids content, 40 and 60 g VS/l.

Keywords: dairy manure, volatile fatty acids, hydrolysis, acidogenesis, methanization.

*****Corresponding author. Tel.: +34-942202286; fax: +34-942201703
E-mail address: ricoc@unican.es

1. Introduction

The recent growth of intensive dairy farming implies the industrialization of dairy farms, improving production efficiencies. However, the rise in the number of cows has led to large quantities of dairy manure, which can not be properly managed by farmers, giving place to environmental damages. There is also a concern regarding green house gases (GHG) emissions. Livestock industry is the largest source of green house gases methane (CH_4) and nitrous oxide (N_2O) in the primary sector in Europe. Emissions of these gases by dairy cattle production systems, accounted approximately 7% of total European Union GHG emissions [1]. Anaerobic conversion of dairy manure into biogas is an attractive way of managing these kinds of wastes, providing benefits such as renewable energy, environmental protection and nutrients recovery [2-7]. Not only environmental benefits are accomplished by anaerobic digestion, a renewed interest in this technology has emerged since new trends to limit carbon dioxide and other GHG emissions, carbon taxes and subsidies of biomass energy would make anaerobic digestion economically competitive [8].

Dairy manure composition depends on the conditions under which animals are kept on the farm (dairy cows, breeding cows, calves). In addition to solid and liquid dejections, the waste collected by means of either mechanical or hydraulic scraping of the floor, can drag other wastes along into the dung pit, so that dairy wastes extracted from cowhouses contain rests of food and bedding (straw, sand, sawdust, etc), being many of these solids not biodegradable or only slowly so. The lignocellulose and hemicellulose within plant fibers creates the most difficulties for anaerobic digestion. Lignin is very resistant to enzyme hydrolysis and microbial degradation, creating a physical barrier preventing hydrolysis of cellulose [9]. Due to high solid concentration, hydrolytic stage is the limiting step in anaerobic digestion of dairy manure.

Separation of liquid and solid fractions of the waste is a desirable upstream operation in the treatment process: dewatering the solid fraction (SF) lowers the cost of shipping and increases the energetic yield if SF is subjected to combustion processes for energy production. In addition, separation is a way to

produce manure fractions with higher gas potentials based on volume, since the water can be drained from the solids, giving these fractions a higher volatile solids (VS) concentration [10]. Moreover, the separated liquid fraction (LF), with lower suspended solids (SS) content will be more easily subjected to anaerobic process in CSTR systems.

Previous experiments to separate liquid and solid fractions with mechanical separators and settling tanks had been performed [11-13]. The SS content in the LF from screening is still high and only a small fraction of its COD is in soluble form [14, 15]. Very low SS concentration is required if the LF is going to be treated in high load anaerobic reactor, involving lower temperature and hydraulic retention time (HRT).

In order to enhance anaerobic digestion for separated LF, manure can be subjected to pre-treatments prior to receiving separation processes, improving later treatment performance. Several studies have been reported concerning pre-treatment technologies for reducing the time requirements for the hydrolysis of insoluble organics, increasing the biogas potential of manure [9, 16-23].

Mechanical maceration of manure reduces the size of particles, having positive effects on the anaerobic digestion of the substrate since size reduction can lead to more rapid digestion. Mechanical maceration has been tested by Angelidaki and Ahring [9] resulting in average increase of the biogas potential of 17%. In another study performed by Hartmann *et al.* [16] different concepts of implementation of mechanical pretreatment for enhancing the biogas potential from fibers in manure feedstock were evaluated by sampling before and after macerators at different biogas plants and from a fiber separation unit, finding an increase of the biogas potential of up to 25% by pretreatment of the whole feed in the macerator before the reactor. Regarding particle size, Palmowski and Müller [17] demonstrated two positive effects of the comminution of organic solids on their biodegradability under anaerobic digestion: improved volumetric gas production and reduction of the HRT.

The use of thermal pretreatment to increase the available soluble organics of sewage sludge prior to anaerobic digestion has been early reported by Haug *et al.* [18]. Thermal treatment solubilises organic solids reforming the sludge property and then increasing methane production. Yoneyama *et al.* [19] studied the thermal pre-treatment for methane fermentation of cow manure in terms of solid-liquid separation and subsequent application of high rate UASB reactor for the treatment of the liquefied supernatant, obtaining 1.3 times higher methane gas production compared to conventional treatment. Bonmatí *et al.* [20] studied the thermal pre-treatment at low temperature (<90ºC) of pig slurry, determining that this treatment at 80ºC for three hours improved the hydrolytic phase of anaerobic digestion.

Other pre-treatment technologies reported to reduce the time requirements for the hydrolysis of particulate organics are: chemical pre-treatment [9, 21], sonication [22] and ozonation [23].

Taking into account the different kinetics of hydrolytic, acidogenic and methanogenic stages, Nozhevnikova *et al.* [24] proposed a two-step anaerobic manure treatment process in which hydrolytic and acidogenic stages were carried out at high temperature, achieving hygienization of manure. Then solid and liquid fractions were separated and finally anaerobic digestion of LF under low temperature conditions was completed. Volatile fatty acids (VFA) are intermediate compounds in the anaerobic process which concentration is one of the most important control tests for detecting instability in the anaerobic digestion process [25].

In this sense, Asinari Di San Marzano *et al.* [26] suggested that controlled storage of manure could act as the first stage of anaerobic digestion systems. Separation of hydrolytic and acidogenic stages from the methanogenic is a fundamental aspect as methanization should take place in the high load reactor treating the liquid fraction. Temperature becomes a main factor, low temperatures decreases the kinetics of both hydrolytic and methanogenic stages, so the combination of this variable and other ones, such as the hydraulic retention time, will provide the optimum conditions to develop the hydrolytic stage without

methanization. Van Velsen [27] determined that biogas production from anaerobic digestion of swine manure greatly diminished below 25ºC, disappearing practically at 15ºC, while Umetsu *et al.* [28] observed negligible methane emissions from dairy manure at 10ºC. On the other hand, Sawyer and McCarty [29] proved that methanogenic biomass suffered a severe inhibition at pH lower than 6.5.

The present work is a part of a research project focused on obtaining a liquid fraction of dairy manure without particulate material and a high percentage of biodegradable chemical oxygen demand (COD_{BD}), in order to be treated in high load anaerobic reactors. The objective of this work was to study the evolution with the time of the characteristics of the liquid fraction of dairy manure with different volatile matter contents (40, 60, 80 g VS/l) at two different temperatures (20, 35ºC) up to 25 days in a controlled dungpit with regards to the development of the hydrolytic and acidogenic stages. Depending on VS concentration and temperature levels, the subsequent separation of liquid and solid fractions could be carried out at the time when the development of hydrolytic and acidogenic stages were maximum before methanogenesis starts up.

2. Materials and methods

2.1. Feed Characteristics

Fresh manure was taken directly from the cow house in a dairy cow farm located in Loredo (Cantabria, North Coast of Spain). Total solids (TS) concentration ranged from 100-140 g TS/l during the investigation work. VS concentration was determined and dilution with tap water was done to obtain three different concentrations (40, 60 and 80 g VS/l) and stored at 4ºC prior to use.

2.2. Batch Experiments

Experiments were performed in batch systems trying to simulate the conditions of a controlled plug flow dung pit. It must be taken into account that batch and plug flows have the same performance equations. Experimental variables were: VS concentration, temperature and HRT; so that the effects due to the development of hydrolytic and acidogenic stages in the characteristic of the LF supernatant could be

studied. The experiment was carried out at 20 and 35ºC for three different VS concentration values, so that six experimental conditions were conducted. A thermostatic bath maintained the selected temperature. The duration of the batch tests was 25 days since this time could be a proper retention time for manure into the dung pit before solid-liquid separation pretreatment and/or anaerobic digestion. Longer retention times would imply bigger size dung pits in addition to the possibility that methanization starts up and the release of volatile organic matter during the hot months of the year.

Five reactors were operated for each experimental condition. One reactor, made of PVC, having a total volume of 2.5 litres, was used to measure the gas production for each experimental condition by means of a gas meter device. The other four reactors were made of polyethylene with 0.5 litres total volume equipped with gassing out tubes in the top to allow the release of gas and to avoid the entrance of air by an overpressure obtained by submerging the end of the tubes in water (2 cm column water).

To know the development of hydrolytic and acidogenic stages for each of the six experimental conditions the polyethylene reactors were opened one at a time on days 5, 10, 15 and 20; the PVC reactor content served as the sample for day 25. That is to say, for each experimental condition one of the polyethylene reactors was opened at day 5 to take the sample and then was eliminated; at day 10 other polyethylene reactor was opened to take the sample and then was eliminated as well. Analogous operation was done with the other two polyethylene reactors and at day 25 the sample was taken from the PVC reactor. Gas production was determined daily. For each experimental condition the PVC reactor was filled with 1750 g of dairy manure and the four polyethylene reactors were filled with 400 g of dairy manure, then tightly closed and placed into the corresponding thermostatic bath. Samples were tested for pH, chemical oxygen demand (COD_{sup}), Total Kjeldahl Nitrogen (TKN) and organic carbon (OC_{sup}) in the supernatant; VFA and ammonia nitrogen ($N\text{-}NH_4^+$) in the soluble fraction. No stirring was applied. Figure 1 represents the reactors employed and the configuration of the reactors into the thermostatic bath for each experimental condition.

To prove the results obtained in the previous test, two new assays were carried out for a period of 60 days. Employing a 25 litres PVC laboratory scale reactor equipped with a stirring device, with 20 litres of useful volume, the evolution of supernatant dairy manure characteristics was tested at 20ºC and 35ºC. The stirred system was only activated immediately before samples were withdrawn.

2.3. Analytical Methods

The samples were allowed to settle for six hours. After settling, the supernatant was decanted for the analysis of pH, COD_{sup}, TKN and OC. To determine VFA and $N\text{-}NH_4^+$ concentrations, the decanted sample was filtrated through a Whatman GF/C 1.2-μm filter, with analyses performed on the filtrate.

COD, VS, TKN, $N\text{-}NH_4^+$ and pH analyses were performed as described in Standard Methods for the Analysis of Waters and Wastewaters [30]. VFA were determined using a HP 6890 GC equipment fitted with a 2m x 1/8 in. glass column, liquid phase 10% AT 1000, packed with the solid support Chromosorb W-AW 80/100 mesh. Nitrogen was the carrier gas and a FID detector was installed. Gas composition was assayed on a 2m Poropak T column in a HP 6890 GC System with helium as carrier gas and TCD detector. Organic Carbon analysis was carried out in a SHIMADZU 5050 OC Analyzer, by combustion-infrared method (5310B) of Standard Methods. VFA concentrations were expressed in COD units.

Methane production was measured by means of a displacement system using an alkaline solution in order to absorb the CO_2 produced. All the measurements are expressed at 20ºC and standard pressure of 760 mm Hg (NCTP). Methane production was converted into COD by the conversion factor at 20ºC and wet conditions, 385 ml CH_4 equal to 1 g COD_{CH4}.

The values for each parameter of all the samples were determined in triplicate; the values shown are the mean ones. When deviation of some of the three determined values in relation to mean value for any parameter was higher than 5%, a new determination was done.

3. Results and discussion

Similar behaviors were found for the three samples of manure with different VS concentration for each experimental temperature conditions. For this reason the following results are refereed to manure samples with 60 g VS/l. Table 1 shows the mean initial characteristics of dairy manure with a VS content of 60 g/l. The initially pH value was 7.5, a suitable value for methanogenic bacterium activity which are in the digestive apparatus of dairy cows, so during the first days of the experiment there was a little methane production, but then pH values decreased and the activity of the methanogenic bacterium was disabled. In Table 1 it can be observed that the initial value for COD due to VFA (COD_{VFA}) was the 42% of the COD on the supernatant obtained by centrifuging the manure sample. The mean values for all the parameters tested during the experiment are shown in Table 2.

At 20ºC pH value decreased rapidly, reaching a value of 6.5 at day 5, then decreased slowly down to 6.3 at day 25. Operation at 35ºC resulted in lower pH values than at 20ºC. At day five, pH went down to 6.3 at 35ºC. At day 15 a minimum value of 6.1 was determined at 35ºC, whereas at 20ºC pH value was 6.2. Starting from day 15, the system working at 35ºC experimented an increase in the pH value, especially the last five days when a slight methanization started, reaching a final value of 6.8; while at 20ºC pH did not increase. In Figure 2 the pH evolution during the experiment for both operational temperatures is shown. The reason for these differences is the faster kinetics for hydrolytic and acidogenic stages at 35ºC compared to 20ºC. In addition the solubility of CO_2 is lower at 35ºC which implies lower buffer capacity. Due to the higher methanogenic bacteria activity at 35ºC, it was probed that samples that worked at 35ºC started methane production at the end of the experimental time.

The initial decrease in pH values implied a reduction of inorganic carbon concentration (IC) from 538 mg/l to 190 mg/l and 156 mg/l for 20ºC and 35ºC respectively. For all the samples, concentration values of IC were higher at 20ºC; however, on day 25 at 35ºC IC concentration was almost the same than that at 20ºC due to the beginning of methanization.

At 20ºC a moderate and continuous increase of COD_{sup} and COD_{VFA} happened as hydrolytic and acidogenic stages were developing. COD_{sup} rose from its initial value, 19,975 mg/l to 25,680 mg/l at day 25, whereas VFA concentration (expressed as COD_{VFA}) increased from 8354 to 17,275 mg/l. Methane production was basically negligible. A similar trend was observed in organic carbon (OC) concentration for the samples stored at 20ºC, going from 7256 mg/l at day 0 to 9243 mg/l at day 25. In Figure 3, the evolution of COD_{sup}, COD_{VFA} and COD_{CH4} with time are shown. At 20ºC a faster increase in VFA concentration over COD_{sup} concentration can be observed, which is in accordance with the higher temperature dependence of hydrolysis kinetics than that of acidogenic. As mentioned before, similar trends were observed at 20ºC for the three systems with concentrations of 40, 60 and 80 g VS/l.

In Figure 3, the changes of COD_{sup}, COD_{VFA} and COD_{CH4} with time of the system at 35ºC are also shown. At this temperature the increase of COD_{sup} and COD_{VFA} was faster than that at 20ºC and in this case the initial increments in COD_{sup} and COD_{VFA} performed similar kinetics. At day 5, COD_{sup} reached practically its highest value, and then hardly increased up to 29,476 mg/l at day 20. From day 20, a decrease in COD_{sup} was detected, coming down to 26,935 mg/l. Previous non published data for dairy manure showed that about 40% of VS are contained in particles larger than 1 mm whereas 45% of the VS are contained in particles smaller than 0.025 mm. The remaining VS are present in the intermediate sizes. That is in agreement with data brought up by Chang *et al.* [31]. Operation at 35ºC allowed a rapid solubilisation of the particulate matter of smaller sizes, but had a little impact on particulate matter of bigger sizes. Something similar happened with COD_{VFA}; a rapid increase during the first five days of the experiment and then a slower raise until day 20. COD_{VFA} reached up to 75% of the CODsup; no inhibition seemed to happen in VFA formation as Chang *et al.* [31] indicated. COD_{VFA} reached a maximum value of 21,204 mg/l at day 20, and then started to decrease as happened with COD_{sup}. These circumstances coincided in time with the starting in methane production at day 25, which reveals that methanogenic stage was beginning to take place after a period of 25 days due to methanogenic biomass growth and the presence of adequate environmental conditions, as variation in pH corroborates.

Similar results were observed for concentrations of organic carbon (OC) values. The COD_{sup}/OC ratios were always next to the theoretical ratio 2.67, it must be taken into account that this value is for the theoretical oxygen demand (TOD) in relation to OC, since COD is approximately 85% of TOD.

Concentrations of organic nitrogen (TKN) on supernatant were also increased at 35ºC, as can be seen on Table 2. This increase in supernatant TKN concentration is caused by two factors: fragmentation of particles and hydrolysis of proteins. The evolution of ammonia nitrogen for ammonification process from TKN for both operational temperatures is represented in Figure 4. At 35ºC $N\text{-}NH_4^+$ concentration rapidly grew over 1400 mg/l, maintaining similar values the rest of the assay. At 20ºC a constant and steady raise in the concentration of ammonia nitrogen was observed, reaching a value of 1295 mg $N\text{-}NH_4^+$/l at day 25.

The predominant VFA was acetic acid and changes in its concentration set the trend for changes in the total VFA concentrations. A decrease in the initial proportion of acetic acid and an increase in the proportion of propionic were observed, as referred by Patni and Jui [32]. From day five on, the distribution of individual VFA as a percentage of the total remained nearly the same during the experiment. On a molar basis acetic acid constituted 65-70% of the total VFA, as Patni and Jui [32] referred as well. Acetic, propionic and butyric acids together accounted for more than 95% of the VFA with mean individual values similar than those found by Hill [33]. Figure 5 shows the concentration of acetic, propionic and butyric acids for both temperatures along the test.

In Figure 6 the evolution of COD_{VFA} (expressed as COD_{VFA}/g VS) at 20ºC and 35ºC for the three experimental concentrations (40, 60 and 80 g VS/l) are shown. At 35ºC similar trends were observed for the 40 and 60 g VS/l samples. However it did not happen for the 80 g VS/l sample. From day five on, the ratio COD_{VFA}/g VS did not increase as the other samples with lower VS content. At 20ºC something similar happened. Evolution of COD_{VFA} for 40 and 60 g VS/l samples followed a similar behavior, except the last days probably due to the experimental errors, whereas the sample with the highest VS

concentration showed a different evolution, with a similar trend than that with the same concentration at 35ºC.

In order to check the operation of the samples operated in discontinuous mode, the 20 useful litre volume reactors were operated at 20ºC and 35ºC. Figure 7 shows the evolution of pH, COD_{VFA} and COD_{CH4} for 60 days at 20ºC and 35ºC. The pH values showed a similar behavior than that found during the previous experiment, when the samples were analyzed each 5 days. At 35ºC the pH value dropped to 6.2, maintaining this value until day 30, when pH raised to 6.7 and methane production started at the same time. From that day on, COD_{VFA} decreased from 23,500 mg/l to 10,800 mg/l at day 60. A similar performance but with a little lag time in methane production compared to that observed previously. With regard to the reactor operating at 20ºC, during the 60 days it was controlled, methane production was not detected whereas COD_{VFA} concentration increased slowly during the first 30 days up to 21,000 mg/l, then maintained close to that value until day 60. The reactor operated at 35ºC reached those levels of COD_{VFA} at day 10.

4. Conclusions

No significant differences in terms of characteristics in supernatants were observed in the behaviour of manures with 40 and 60 g VS/l for each of the operating temperatures. Manure with 80 g VS/l showed a different behavior compared with the samples with lower VS concentration at both operating temperatures. A similar degree of hydrolysis and acidogenesis was achieved at 35ºC and 20ºC at 25 day. As a matter of fact, due to faster kinetics at 35ºC the concentrations of supernatant COD, COD_{VFA}, TKN and $N\text{-}NH_4^+$ rapidly increased at 35ºC in the first five days but then hardly increased during the rest of the days, whereas at 20ºC the raise of these parameters was slow and steady during the 25 days of the experiment. Maximum values por COD_{sup} and COD_{VFA} were similar for samples stored at 35 and 20ºC, but obtained at days 10 and 25, respectively. At 35ºC, and after a period of 25 days pH values and methanogenic bacterium activity were suitable for the beginning of methanization for manure with 40 and 60 g VS/l. This behaviour was the same in the 25 litres reactor operated with 60 g VS/l dairy manure for

both 20 and 35ºC temperatures. However, manure with 80 g VS/l did not start the methanogenic stage probably due to a lack of suitable pH values.

The performance of a controlled dung pit to develop the hydrolytic and acidogenic stages in a manure based biogas plant would be highly dependant on the operational temperature. At ambient temperature (20ºC) dairy manure can be stored in a plug flow dungpit at least during 60 days, taking place the hydrolytic and acidogenic stages without methanization. At 35ºC, the HRT for dairy manure in a dung pit should be lower than 25 days because methanization could take place before entering the anaerobic reactor reducing the biogas potential.

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6. References

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